Identifying Negative Control Chemicals for use in Larval Zebrafish Behavior Assays

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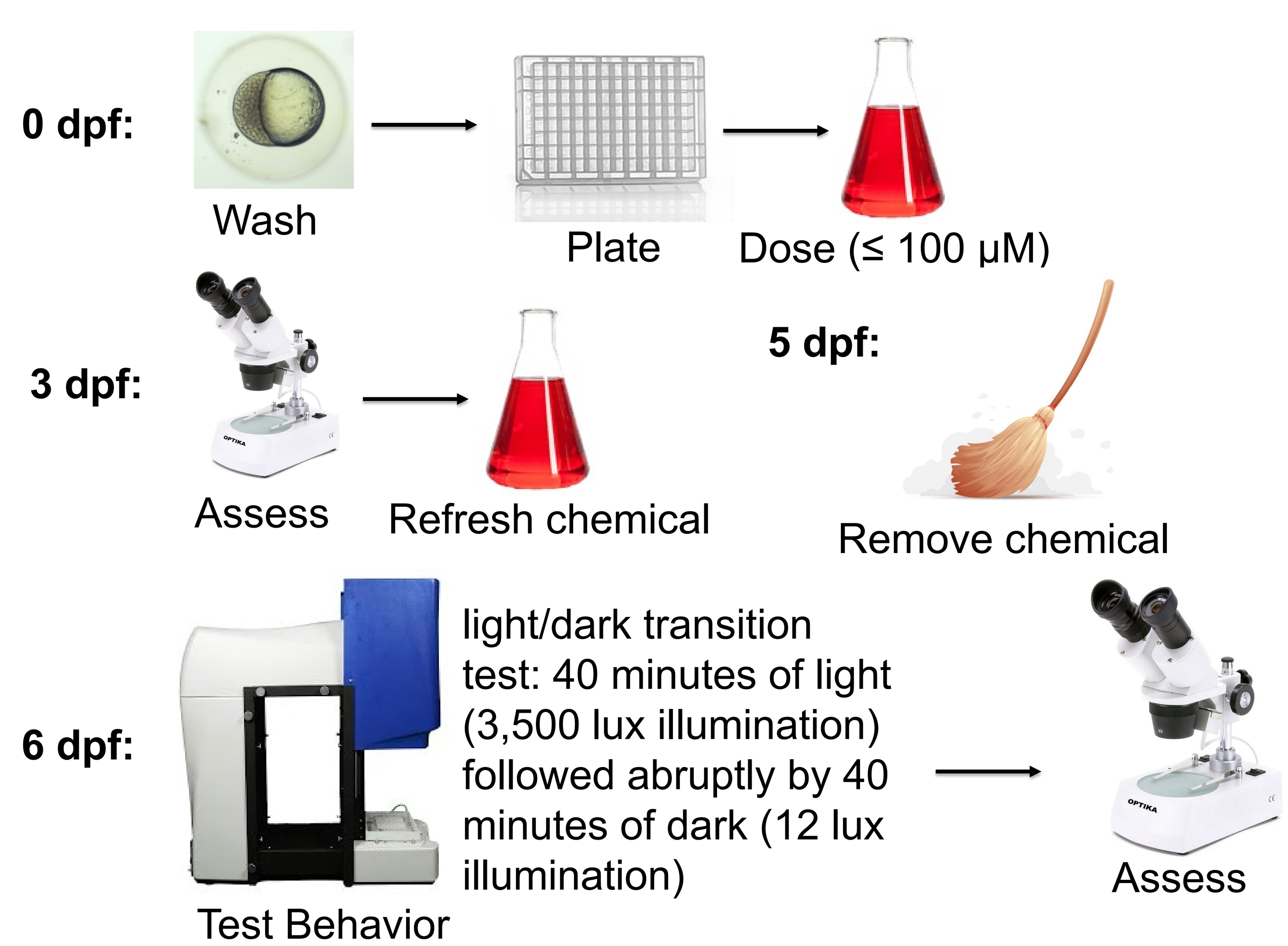
Background

- We assess the developmental neurotoxicity potential of chemicals using a medium-throughput larval zebrafish screening assay which measures larval zebrafish locomotor behavior in response to visual stimuli.
- Little research has been done to identify chemicals that can serve as reliable negative controls in a larval zebrafish behavior assay.
- Positive and negative control compounds allow researchers to measure the sensitivity and specificity of their assays.
- Martin and coworkers (PMID: 35908584) found 9 candidate negative control chemicals for developmental neurotoxicity testing given that they had been shown in the literature not to induce developmental neurotoxicity in mammals.
- **Purpose:** Identify negative control chemicals for use in larval behavior assays.

Methods

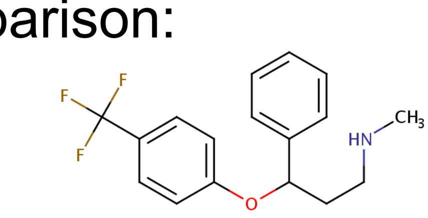
Step 1: assess the lethality and teratogenic potential of each chemical by performing a dose range-finding developmental toxicity study up to 100 µM

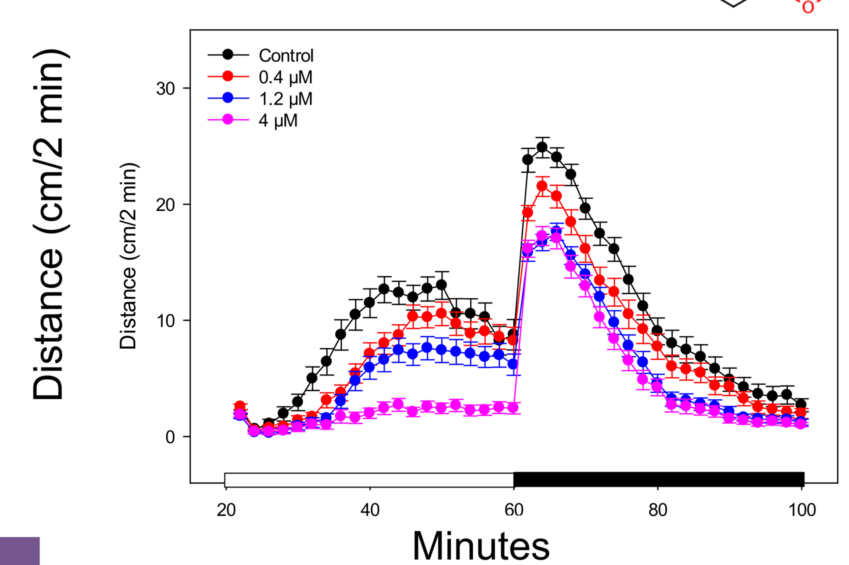
Step 2: select non-developmentally toxic concentrations of each chemical and perform locomotor behavior assay at 6 days post fertilization (6 dpf)



Results: Evidence Suggests that All Chemicals Tested are Potential Negative Controls for Larval Zebrafish Behavioral Assays

Tested for comparison:
Fluoxetine
(positive control)



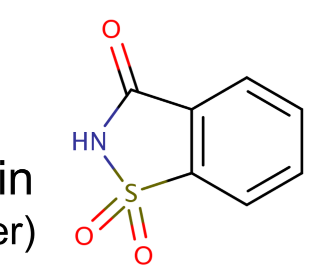


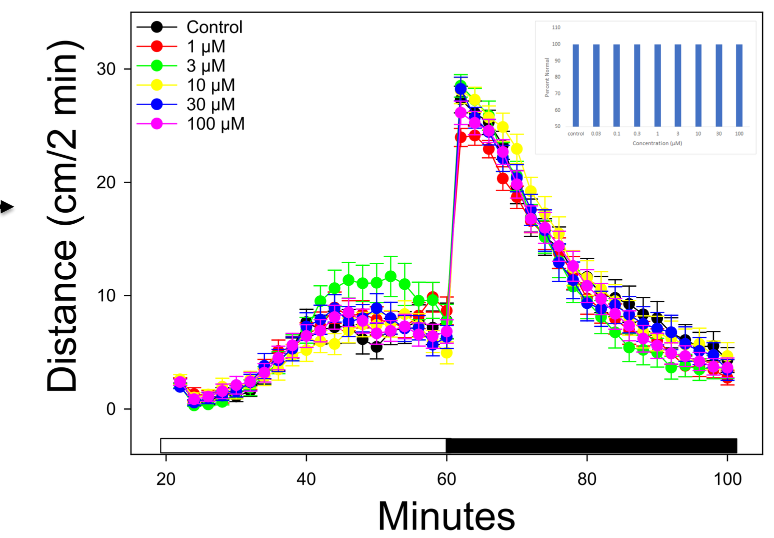
vs.

At 4 µM, 1.2 µM, and 0.4 µM, behavior was affected.

Overall effect of Fluoxetine: $p < 0.001$
Overall effect of time: $p < 0.001$

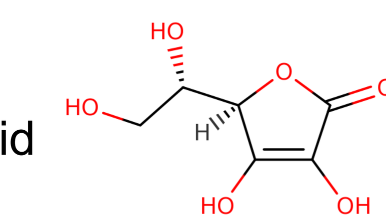
Saccharin
(Sweetener)

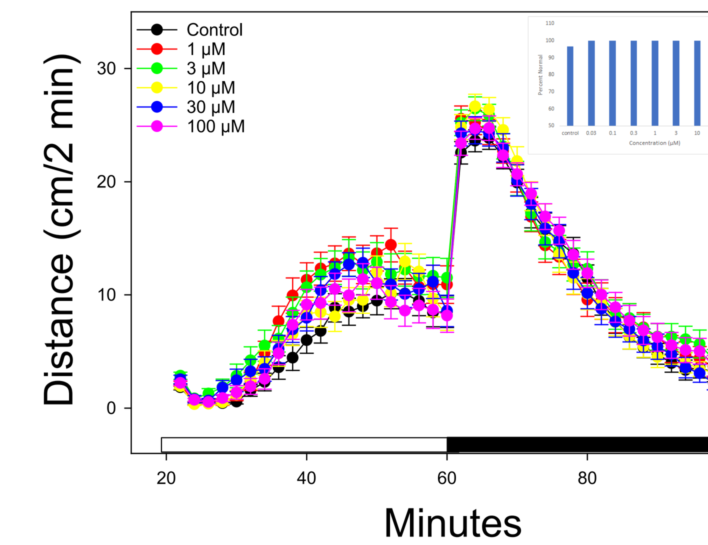




Overall effect of Saccharin: $p = 0.98$
Overall effect of time: $p < 0.001$

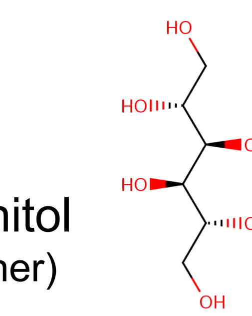
L-Ascorbic Acid
(Vitamin C)

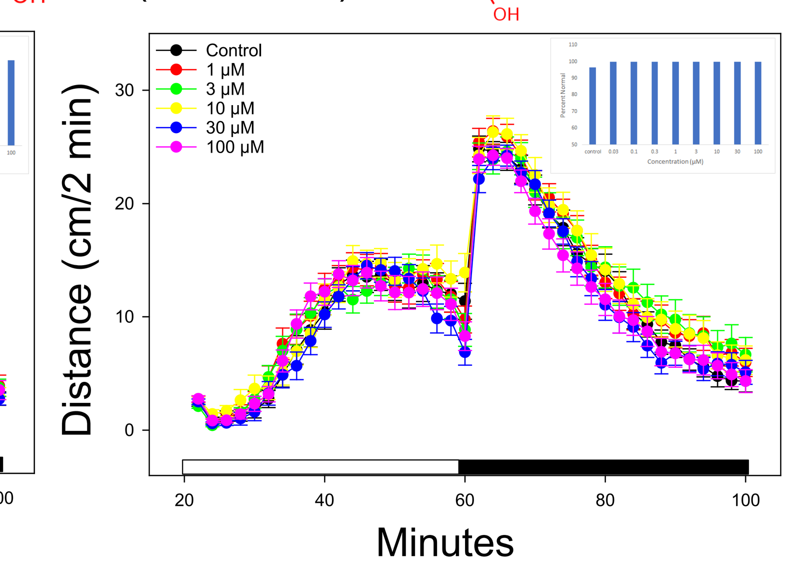




Overall effect of L-Ascorbic Acid: $p = 0.26$
Overall effect of time: $p < 0.001$

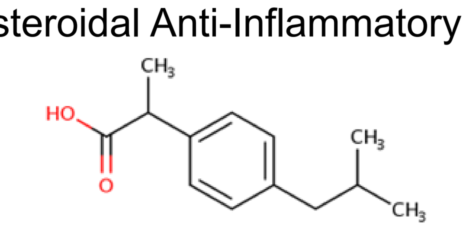
D-mannitol
(Sweetener)

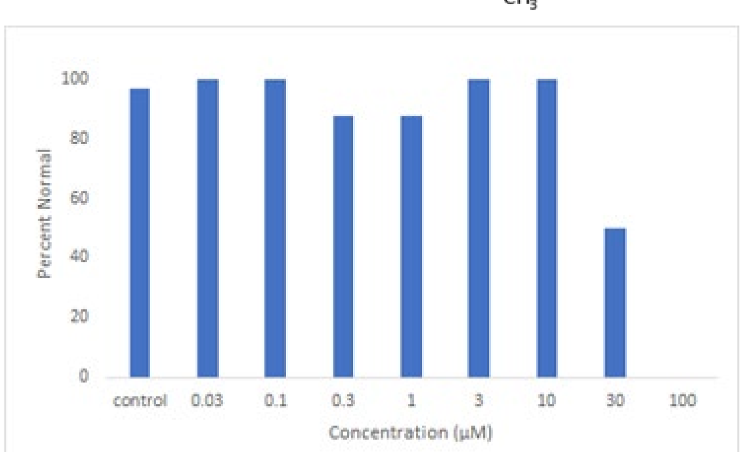




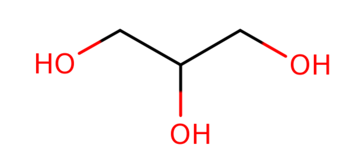
Overall effect of D-mannitol: $p = 0.38$
Overall effect of time: $p < 0.001$

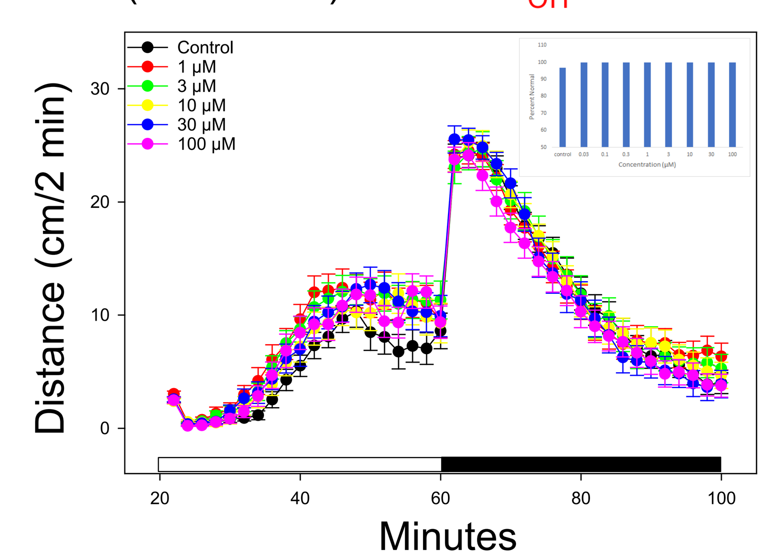
Ibuprofen
(Nonsteroidal Anti-Inflammatory Drug)





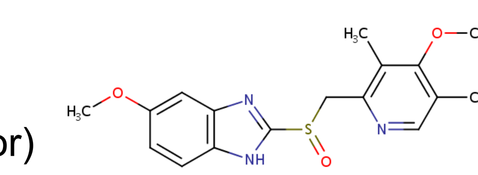
Glycerol
(Sweetener)

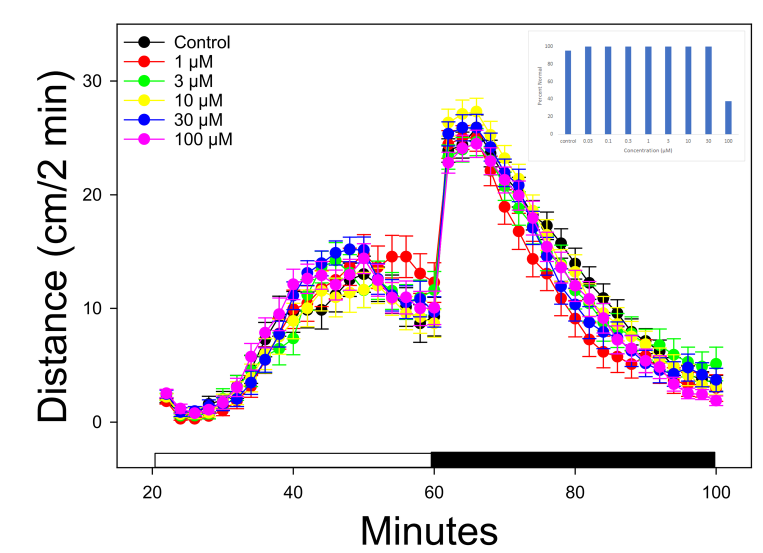




Overall effect of Glycerol: $p = 0.73$
Overall effect of time: $p < 0.001$

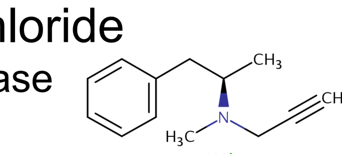
Omeprazole
(Proton-Pump Inhibitor)

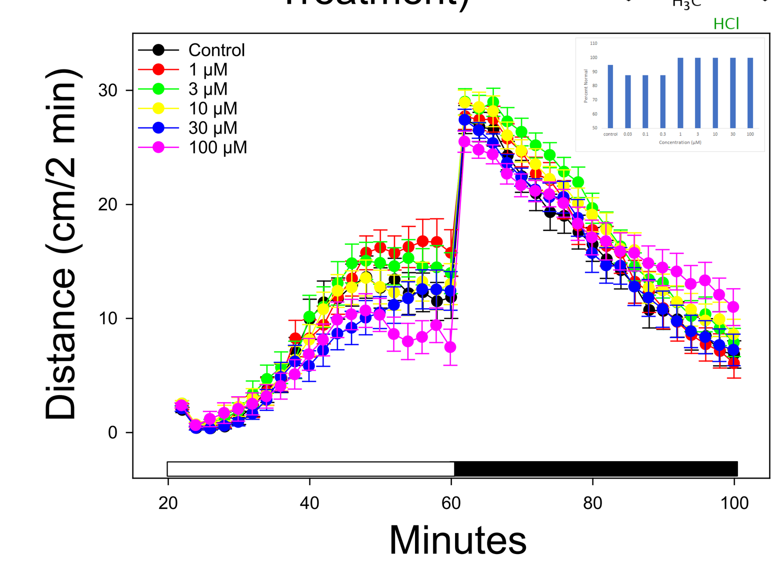




Overall effect of Omeprazole: $p = 0.97$
Overall effect of time: $p < 0.001$

Selegiline Hydrochloride
(Parkinson's Disease Treatment)





Overall effect of Selegiline HCl: $p = 0.13$
Overall effect of time: $p < 0.001$

- None of the chemicals, except for ibuprofen (100 µM and 30 µM) and omeprazole (100 µM), caused developmental toxicity.

Further Research

- Continue to identify more chemicals that could serve as negative controls for the larval zebrafish locomotor assay.
- Incorporate these negative chemicals in chemical libraries to improve sensitivity and specificity of screening for developmental neurotoxicity.

Acknowledgements

We thank those responsible for providing excellent oversight, maintenance, and upkeep of zebrafish facility. Disclaimer: *The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.*